Original Article
Effects of heme oxygenase-1 on cytokines and histological changes of pancreas and liver in rats with severe acute pancreatitis

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Abstract: Background: Recent studies indicate that heme oxygenase-1 (HO-1) is capable of protecting cells through the mechanism of anti-oxidation, maintaining microcirculation and anti-inflammatory, etc. Objective: We aim to investigate the effects of HO-1 in pancreas and liver on severe acute pancreatitis (SAP) rats and explore its probable mechanism. Methods: A total of 40 male SD rats were randomly divided into 4 groups: control group (n=10); SAP group (n=10); HO-1 stimulation group (75 μg/kg hemin was injected intraperitoneally 30 minutes after induction of SAP, n=10) and HO-1 inhibition group (20 μg/kg ZnPP was injected intraperitoneally 30 minutes after induction of SAP, n=10). 24 h after induction of SAP, the histopathological changes of pancreas and liver tissues were observed, and the HO-1, IL-10 and TNF-α level of serum, pancreas and liver tissues were detected. Results: Comparing to SAP model group, the pathological scores of pancreas (7.50±0.58 vs. 10.50±0.71, P<0.05) and liver (1.20±0.42 vs. 1.70±0.48, P<0.05) were significantly decreased in HO-1 stimulation group; the concentrations of HO-1 (0.97±0.02 ng/ml vs. 0.83±0.02 ng/ml, 0.78±0.09 ng/ml vs. 0.56±0.12 ng/ml, 0.73±0.05 ng/ml vs. 0.59±0.02 ng/ml, P<0.05) and IL-10 (101.72±2.63 pg/ml vs. 72.77±4.20 pg/ml, 63.58±1.02 pg/ml vs. 53.57±4.17 pg/ml, 169.40±3.06 pg/ml vs. 160.30±7.03 pg/ml, P<0.05) of serum, pancreas and liver tissues were significantly increased, whereas the concentration of TNF-α (22.85±1.74 pg/ml vs. 28.00±0.81 pg/ml, 26.50±1.3 pg/ml vs. 32.48±4.96 pg/ml, 35.88±0.98 pg/ml vs. 43.22±1.11 pg/ml, P<0.05) were significantly decreased in HO-1 stimulation group. Compared to SAP model group, the pathological scores of pancreas (7.50±0.58 vs. 10.50±0.71, P<0.05) and liver (1.20±0.42 vs. 1.70±0.48, P<0.05) were increased in HO-1 inhibition group; the concentrations of HO-1 (0.76±0.05 ng/ml vs. 0.83±0.02 ng/ml, 0.39±0.06 ng/ml vs. 0.56±0.12 ng/ml, 0.52±0.01 ng/ml vs. 0.59±0.02 ng/ml, P<0.05) and IL-10 (63.10±2.65 pg/ml vs. 72.77±4.20 pg/ml, 50.33±4.23 pg/ml vs. 53.57±4.17 pg/ml, 148.02±1.88 pg/ml vs. 160.30±7.03 pg/ml, P<0.05) of serum, pancreas and liver tissues were significantly decreased, whereas the concentration of TNF-α (33.52±0.66 pg/ml vs. 28.00±0.81 pg/ml, 34.90±1.31 pg/ml vs. 32.48±4.96 pg/ml, 55.46±1.40 pg/ml vs. 43.22±1.11 pg/ml, P<0.05) were significantly increased in HO-1 inhibition group. Conclusions: The results of the study demonstrated that HO-1 over-expression had protective effects on the pancreas and liver in SAP. Down-regulated expression of TNF-α and up-regulated expression of IL-10 might contribute to its potential mechanism.

Keywords: Heme oxygenase-1, severe acute pancreatitis, pathological scores, cytokines

Introduction

Acute pancreatitis (AP) is a common inflammatory process with great variability in severity [1]. It runs a self-limiting course in most patients; however, 10% to 20% of all cases demonstrate severe acute pancreatitis (SAP) [2, 3]. The pathogenesis of SAP is complicated and it is not only located in the pancreas but also involve many other organs. Studies have indicated that the explosive production and release of pro-inflammatory cytokines play an important role in its pathogenesis [4]. In the early phase of inflammatory response, pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1), released by mononuclear phagocyte, neutrophil and infected pancreas, can trigger systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS) [5]. Moreover, approxi-
Effects of heme oxygenase-1 on rats with severe acute pancreatitis

Approximately 50 percent deaths occur within the first week of the attack, because of the SIRS and MODS being triggered in the later course of the disease [6, 7]. Thus, in the early phase, inhibiting the synthesis of pro-inflammatory cytokines and altering the balance between pro- and anti-inflammatory cytokines might significantly affect the severity of pancreatitis and the survival rate [8, 9].

HO-1 is a 32-kDa member of the stress protein super family and has a broad spectrum of inducers, including metals, nephrotoxins, cytokines, endotoxin, oxidants and vasoactive compounds [10]. Recent studies suggested that overexpression of the HO-1 gene, either by targeted gene transfer or by pharmacological modulation, may lead to significant new and beneficial therapeutic approaches to alleviating and eventually eliminating the oxidative cell damage that occurs in some disease states [11]. These findings suggested that administration of HO-1 could be useful in regulating the pro- and anti-inflammatory cytokines during the initiation of SAP.

In the present study, we focused on the pathological changes of pancreas and liver caused by different expressing status of HO-1 as well as its regulating effect on pro-inflammatory cytokines and anti-inflammatory cytokines in the SAP rats.

Material and methods

Animals

The protocol for this study was approved by the Faculty of Medicine and Health Sciences Ethics Committee for Animal Research, Affiliated Hospital of Shandong University of Traditional Chinese Medicine. All surgery was performed under diethyl ether anesthesia. 40 SD rats (220g-260 g, male, 6-7 week, SPF, provided by Shanghai Experimental Animal Center of Chinese Academy Science) were housed individually and maintained under standard conditions in hanging cages in rooms maintained at 21±1°C using a 12-hour light/dark circle. The animals were fed a regular rat chow.

Induction of severe acute pancreatitis

SAP was induced by the classic method of retrogradely injection of 3% sodium cholate into the pancreatic duct. Briefly, 3% sodium cholate (0.1 mL/100 g, Sigma, USA) was retrogradely injected into the pancreatic duct by microinfusion pump and the pressure maintained for 5min. After the induction, the rats are free to water. 40 SD rats are randomly divided into 4 groups: control group (n=10), SAP group (n=10), HO-1 stimulation group (n=10), HO-1 inhibition group (n=10). The rats of HO-1 stimulation group were treated by intraperitoneal injection of Hemin (75 μg/kg, Sigma, USA) [12] 30 min after the induction of SAP, and the ones of HO-1 inhibition group were injected by ZnPP (20 μg/kg, Sigma, USA) [13] at the same time.

Histological assessment

Pancreas and liver samples were fixed in 40 g/L buffered formaldehyde and embedded in paraffin. Then the samples were cut into 5 μm thick sections, and stained with haematoxylin and eosin for light microscopic examination. Histological assessment was performed by an investigator blinded to the treatment group. The pathological scores of pancreas and liver samples were determined by the standards of Schmidt etc. [14] and Camargo etc. [15].

Measurement of pro-cytokines and anti-cytokines

The rats were anesthetized by diethyl ether 24 h after the induction of SAP, and the blood, obtained from abdominal aorta, was treated by centrifugation in order to get the serum in which the level of HO-1, IL-10 and TNF-α were tested by enzyme immunoassay kit (EIAabTM, China) referring to the manufacturer’s suggesting protocol. The liver and pancreas tissues were removed 24 h after the induction of SAP and were ground into tissue homogenate (1 g+500 μl PBS) which were treated by centrifugation to get the supernatant in which the level of HO-1, IL-10 and TNF-α were tested by enzyme immunoassay kit (EIAabTM, China) referring to the manufacturer’s suggesting protocol.

Data analysis and statistics

Data was analyzed using the SPSS13.0 statistical software (SPSS for Windows, Germany) and it was presented as means ± SEM. Differences between groups were tested by One-Way ANOVA. P<0.05 were considered significant.
Results

Histopathological evaluation and scores of pancreas

The pancreatic pathological changes were observed by optical microscopy. The structure of pancreatic duct in control group shows morphologically normal, whereas the pancreas in SAP group display partly hemorrhage, necrosis and infiltration of neutrophilic granulocyte, and comparing to which, the pancreas of HO-1 stimulation group shows a more relieved pathological damage including the integrity of pancreatic duct and less infiltration of neutrophilic granulocyte, and the pancreas of HO-1 inhibition group were observed with more severe pathological damage which includes large scale pancreatic and vascular necrosis as well as mass infiltration of neutrophilic granulocyte (Figure 1). The pathological scores is significantly reduced as a result of the intervention of HO-1 (10.50±0.71 vs. 7.50±0.58, P<0.05), whereas enhanced as a result of the lack of HO-1 (10.50±0.71 vs. 13.00±0.62, P<0.05) (Table 1).

Histopathological evaluation and scores of liver

The hepatic cells in control group, showing morphologically normal, were observed in cord-like arrangement, and the structure of hepatic lobe is clear. In SAP group, the cytoplasm became

Table 1. Pathological scores of pancreas and liver in each group

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control group</th>
<th>SAP group</th>
<th>HO-1 stimulation group</th>
<th>HO-1 inhibition group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas</td>
<td>0</td>
<td>10.50±0.71b</td>
<td>7.50±0.58a</td>
<td>13.00±0.62a</td>
</tr>
<tr>
<td>Liver</td>
<td>0</td>
<td>1.70±0.48a</td>
<td>1.20±0.42a</td>
<td>2.60±0.52a</td>
</tr>
</tbody>
</table>

Data are presented as means ± SEM (*P<0.05 vs. SAP; **P<0.05 vs. control). N=10 animals per group.

Figure 1. Pathological changes of pancreas (HE ×400). In control group (A) pancreatic lobe manifested structurally clear without congestion and necrosis; In SAP group (B), mass RBC exudation and necrosis could be observed, which relieved in HO-1 simulation group (C) and aggravated in HO-1 inhibition group (D).
Effects of heme oxygenase-1 on rats with severe acute pancreatitis

Figure 2. Pathological changes of liver (HE ×400). In control group (A) hepatocytes were in cord-like arrangement and the hepatic lobe is structurally clear without congestion and degeneration; In SAP group (B) mass RBC could be observed among hepatic cords, which became more relieved in HO-1 simulation group (C) whereas more aggravated accompanying with mass vacuolar degeneration in HO-1 inhibition group (D).

Figure 3. Histopathological scores of pancreas and liver in SAP group, HO-1stimulation group and HO-1inhibition group (control group = 0). Data are presented as means ± SEM (*P<0.05 vs. SAP) N=10 animals per group.

loosened, and the Kupffer cell proliferated in hepatic sinusoid, comparing to which, the hepatic cell in HO-1 stimulation group shows more normal morphology and less Kupffer cells in sinusoid, whereas in HO-1 inhibition group, the hepatocyte behaved spotty necrosis with more loosened cytoplasm and lymphocyte infiltration (Figure 2). The pathological scores is significantly reduced as a result of the intervention of HO-1 (1.70±0.48 vs. 1.20±0.42, P<0.05), whereas enhanced as a result of the lack of HO-1 (1.70±0.48 vs. 2.60±0.52, P<0.05) (Table 1) (Figure 3).

Effect of heme on expression of HO-1, IL-10 and TNF-α in serum, pancreas and liver

Comparing to SAP group, the concentrations of HO-1 (0.97±0.02 ng/ml vs. 0.83±0.02 ng/ml,
Effects of heme oxygenase-1 on rats with severe acute pancreatitis

Table 2. Expression of HO-1, IL-10 and TNF-α of serum, pancreas and liver in each group 24 h after the induction of SAP

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>SAP group</th>
<th>HO-1 stimulation group</th>
<th>HO-1 inhibition group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HO-1 (ng/mL): Serum</strong></td>
<td>0.25±0.04</td>
<td>0.83±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.97±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.76±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.15±0.01</td>
<td>0.56±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.78±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.39±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver</td>
<td>0.31±0.04</td>
<td>0.59±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.73±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.52±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>IL-10 (pg/mL): Serum</strong></td>
<td>10.77±1.96</td>
<td>72.77±4.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>101.72±2.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.10±2.65&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pancreas</td>
<td>27.43±0.82</td>
<td>53.57±4.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.58±1.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.33±4.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver</td>
<td>82.85±7.65</td>
<td>160.30±7.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>169.40±3.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>148.02±1.88&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>TNF-α (pg/mL): Serum</strong></td>
<td>13.60±1.56</td>
<td>28.00±0.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.85±1.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.52±0.66&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pancreas</td>
<td>13.19±1.46</td>
<td>32.48±4.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.50±1.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.90±1.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver</td>
<td>20.47±2.50</td>
<td>43.22±1.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.88±0.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.46±1.40&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are presented as means±SEM (<sup>a</sup>P<0.05 vs. SAP; <sup>b</sup>P<0.05 vs. control). N=10 animals per group. SAP: Severe Acute Pancreatitis.

Discussion

Heme oxygenase (HO) in concert with NADPH-cytochrome P450 reductase catalyzes the oxidation of heme, resulting in the stereospecific cleavage of heme at the α-methane bridge into equimolar amounts of carbon monoxide (CO), iron and biliverdin; the latter is rapidly reduced by the cytosolic enzyme biliverdin reductase to bilirubin. Three isoforms of HO have been identified. HO-2 is the constitutive glucocorticoid inducible isozyme. HO-3 has been cloned only in rats and is not expressed in humans, thus is of no clinical significance [11, 16]. HO-1 is a 32-kDa member of the stress protein super family and has a broad spectrum of inducers, including metals, nephrotoxins, cytokines, endotoxin, oxidants and vasoactive compounds [10]. Recent studies showed that HO-1 is able to protect the cell through the mechanism of anti-oxidation, anti-inflammatory and maintaining normal function of microcirculation in the condition of severe acute pancreatitis.
Effects of heme oxygenase-1 on rats with severe acute pancreatitis

stress [17-19]. The expression of genes responsible for oxidative stress, especially HO-1 [20], is remarkably upregulated in the course of SAP, which suggests the existence of an compensatory mechanism against stress. However, such mechanism is not capable to cope with the overexpression of inflammatory genes, so the pathological damage still precedes in pancreas. As a result, enhancing the expression of HO-1 gene by exogenous approach may benefit to the inhibition of pro-inflammatory cytokines releasing with the purpose of protecting organ functions.

Our primary study showed the highest expression of HO-1 induced by hemin presented 24 h after the induction of SAP, which was selected as the inducing period as a result. This study demonstrated the high expression of HO-1 can ameliorate the pathological injuries of pancreas and liver in SAP, which could be deteriorated due to the inhibition of HO-1 expression. It suggested that the expression of HO-1 correlated with the pathological injuries of pancreas and liver closely and can protect the organ function significantly in SAP.

SAP was characterized by enzyme activation, interstitial edema, hemorrhage and necrosis [21]. Many pro- and anti-inflammatory cytokines are involved in the initiation of SAP, such as TNF-α and IL-10. TNF-α, secreted by activated macrophage and lymphocyte, plays an important role in the occurrence and development in SAP [22], and it can induce the overexpression of IL-6, IL-8 as well as itself, causing the inflammatory cascade and the uncontrolled releasing of inflammatory mediators [23]. IL-10, produced by TH2 cells, macrophages, stellate cells and hepatocytes, has been reported to play an important role in inflammatory diseases [24], and it can inhibit the synthesis of pro-inflammatory cytokines such as IL-2, IL-3 and TNF-α and prevent MODS caused by SAP [4, 25]. In the course of SAP, pro-inflammatory cytokines such as TNF-α are released at first by macrophage in pancreas which leads to the worsening of pancreatitis, and in the meantime IL-10 is released but it is not qualified for the prevention of excessive systemic inflammatory response and pancreatic necrosis which give rise to the overexpression of pro-inflammatory cytokines in remote organs such as liver and kidney [26], and in the end the incidence of MODS is unavoidable [5]. Therefore, the inhibition of TNF-α and promotion of IL-10 are in favor of preventing the local inflammatory lesion of pancreas, prevent MODS and improve survival.

Our study showed the high expression of HO-1 is correlated with the high expression of IL-10 and low expression of TNF-α in serum, pancreas and liver. At the same time, the low expression of HO-1 is correlated with the low expression of IL-10 and high expression of TNF-α. Thus, we reckoned that HO-1 can protect the organs through the mechanism of down-regulating TNF-α expression and up-regulating IL-10 expression. Heme oxygenase-1 will be a potential therapeutic target to patients with SAP [27].

Generally speaking, HO-1 can take positive effect on controlling systemic inflammatory response and protecting organ function, but its more explicit mechanism on the protection of organ function in SAP need further research.

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Effects of heme oxygenase-1 on rats with severe acute pancreatitis

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Disclosure of conflict of interest

None.

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Effects of heme oxygenase-1 on rats with severe acute pancreatitis


